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RESEARCH ARTICLE

MOLECULAR DETECTION OF CARBAPENEMS RESISTANCE GENES IN PSEUDOMONAS AERUGINOSA ISOLATED FROM DIFFERENT HOSPITALS IN NAJAF, IRAQ

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ABSTRACT

Carbapenems antibiotics are an effective treatment option for serious infections, especially that caused by Gram-negative bacilli excluding *Pseudomonas aeruginosa* strains. Significantly increasing the resistance rate to carbapenems may reduce the choice of these effective antibiotics. Mainly the production of carbapenemes (carbapenem-hydrolysis enzymes) causes resistance to these antibiotics. This study was aimed to detect the occurrence of bla_{IMP}, bla_{VIM} (class A), bla_{KPC} (class B) and bla_{OXA-40} and bla_{OX-50} (class D) genes in *P. aeruginosa* carbapenem-resistant isolates recovered in Najaf-hospitals. From various clinical samples, we identified fifty two isolates as *P. aeruginosa*. Thirteen of the isolates were non-susceptible to carbapenems. Of these were carbapenemase positive in nine isolates. The most frequent gene was found bla_{OXA-50} followed by bla_{VIM}, and bla_{KPC}. Coexistence of bla_{OXA-50} and bla_{VIM} was found in 4 isolates, and not detect of bla_{IMP} and bla_{OXA-40} genes. All isolates that the carbapenem resistant genes had an extensive drug resistant (XDR) phenotype. In a conclusion, currently widely occurrence of *P. aeruginosa* carbapenemase genes in Najaf hospitals, this risk in the increase the spreading of XDR isolates in the hospitals. In addition this is the first report of KPC β-lactamase producing by *P. aeruginosa* in Iraq.

INTRODUCTION

Pseudomonas aeruginosa is the most common an prevalent opportunistic human pathogen, Gram-negative bacterium increasingly found in nosocomial infections (1). Infections by this bacteria are hard to treat that due to of high intrinsic resistant to numerous antibiotics or because it ability develops acquired genes of resistance, either by chromosomal encoded mutation genes or by horizontal transfer the gene of antibiotic resistance (2,3). Carbapenems are β-lactams class, including meropenem and imipenem, are the most effective and the widest action of against this organisms isolated in patients (4). However, resistance to carbapenems has emerged by different mechanisms; include modifications in outer membrane permeability and efflux system regulation linked with AmpC β-lactamases overexcited production (5). The β-lactamase areclassification as class A, B, and D, Carbapenemases are associated with its classes (6). Since over two decades ago, first discoveries of Class A carbapenemases, their it infrequently in clinical strains. Class A carbapenemases included three types of include, the SME and IMI are chromosomal encoded and can be induced in response to cefoxitin and imipenem, and KPC carbapenemase enzymes are plasmid encoded. The KPC in a *K. pneumoniae* strain was first identified in United States at 1996 (7,8). Class B or metallo-beta-lactamases (MBLs) are included the VIM, GIM, NDM, IMP and SIM β-lactamases genes, which are placed within a integron, their is diversity of structures, and they have been integrated as gene cassettes. When these integron linked with transposons or plasmids, it there transfer to other bacteria is readily (9). Class D or CHDLs (carbapenem-hydrolyzing class D -β-lactamases) have been exposed more frequently in *A. baumannii* isolates, but has been detected OXA-40 in *P. aeruginosa* (10,11,12), in addition OXA-50 has been observed in several isolates of *P. aeruginosa* as part of the natural component chromosomal of β-lactamases enzymes in that species, but their may not be expressed in all strains and may not cause manifest carbapenem resistance (13).

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In Najaf, little evidence as regards of carbapenem resistant genes in *P. aeruginosa*. This study was aimed at evaluating the occurrence of Ambler classes A, B, and D carbapenemase in *P. aeruginosa* clinical isolates.

MATERIALS AND METHODES

Bacterial Isolates: This study was conducted between Augusts to end of October 2013 in the three main hospitals in Najaf. A total of 996 clinical samples is collected from patients with assumed infections of (midstream urine, blood, burns, ear, sputum, high vaginal, faces, pleural fluid aspirates, CSF and semen) succumbed for routine bacterial studies were collected and analyzed for *P. aeruginosa* isolates detected. Initial diagnosis of isolates made based on morphology of colony on MacConkey agar and King A agar (Himedia, India), Grams staining, odor of cultures and oxidase test. Than the isolates of *P. aeruginosa* were biochemically confirmed with VITEK2-automated system (BioMérieux Company).

Antibiotic Susceptibility Assays: Antibiogram testing was executed by automated VITEK 2-compact system assayed the MIC technique by means of AST-N222 cards. These cards contain the following antibiotics; Piperacillin, Ticarcillin, Piperacillin-Tazobactam, Ticarcillin-Clavulanic acid, Cefepime, Ceftazidime, Imipenem, Meropenem, Amikacin, Tobramycin, Gentamicin, Pefloxacin, Ciprofloxacin and Colistin. Antimicrobial susceptibility testing was done according to the standard CLSI (2013) guideline.

PCR Amplification: DNA was obtained from the isolates by salting out method (Sambrook *et al.*, 1989). A monoplex PCR to amplified the encoding carbapenemases genes, was run using the primers of bla_{KPC} (class A carbapenemase), bla_{IMP}, bla_{VIM} (class B metalo-β-lactamase), bla_{OXA-40}, bla_{OXA-50} and (class D carbapenemase) (Table 1). The amplification conditions were carried out on protocol was used depending on BIONEER PCR PreMix manufacturer's instruction, and analyzed their amplified products by gel electrophoresis on a 1.5% agarose gel and ethidium bromide staining using gel documentation system (Biometra, Germany). DNA ladder 100 bp was used as a DNA molecular weight marker (Bioneer, Korea).

RESULTS

Of 52 *P. aeruginosa* were detected in burn 20 (38.4%), followed by ear 17 (32.7%), urine 10 (19.2%), blood (body fluids) 4 (7.7%) and vaginal 1 (2%) (Fig. 1).

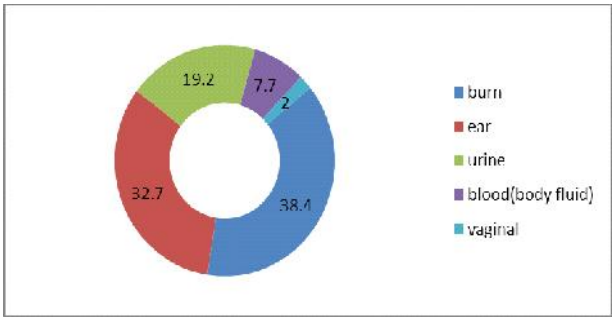

Figure 1. Percentage of *P. Aeruginosa* isolates according to sources

According to MIC measurement of *P. aeruginosa* isolated from clinical samples (Table 2), the susceptibility appears resistance to colistin 3.8%, the most effective (only 2 isolates resist), fallow the resistance value for meropenem 21.2% (11 isolates) and imipenem 25% (13 isolates). Also observed the resistant phenotypes for the β -lactam (Ticarcillin 50%, Ticarcillin-clavulanic acid 44.2%, Pipracillin 40.4%, Piperacillin-tazobactam 36.5%, Ceftazidime 36.6%, Cefepime 30.8%), but for aminoglycoside antibiotics (amikacin 23%, tobramycin 23.7%, gentamicin 44.2%), and for quinolones antibiotics (ciprofloxacin 42.3% and pefloxacin 44.2%). Our finding 13 (25%) of the isolates were categorized as non-susceptible to carbapenems antibiotics. Of these, 11 isolates exhibited completely resistant to both imipenem and meropenem (MIC ≥ 16µg/ml), while two isolate appeared completely resistant to imipenem (MIC ≥ 16µg/ml), but exhibited intermediate resistant to meropenem (MIC = 8µg/ml). All carbapenem resistance isolates were obtained from burn wound. All carbapenem resistance isolates were subjected to PCR for the presence of five carbapenemase resistant genes tested. Results revealed that only 9 (69.2%) isolates possessed at least one gene. Three of the five genes found alone or in combination (Table 3). The bla_{OXA-50} gene was harbored by 7 (53.8%) of the isolates (Fig. 2- A and B). In addition, the bla_{VIM} gene was detected in 5 (38.4%) isolates (Fig. 3- A and B) and only one isolate was found to harbor the bla_{KPC} gene (Fig. 4). Coexistence of bla_{OXA-50} and bla_{VIM} was detected in 4 (30.8%) isolates. All isolates were negative for bla_{IMP} and bla_{OXA-40} type genes. A particularly important feature was that all isolates harboring carbapenem resistant genes had an XDR resistant phenotype. These isolates were completely resistant to penicillins (piperacillin and ticarcillin), β-lactam/β-lactamase inhibitor combinations (piperacillin-tazobactam and ticarcillin-clavulanic acid), gentamicin. Additionally, the majority of these isolates exhibited resistance to cefepime, ceftazidime, tobramycin, amikacin, ciprofloxacin, and pefloxacin. The most active antipseudomonal agents against isolates were colistin with 100% susceptibility.

DISCUSSION

Carbapenems are used as last choice drugs for the treatment of infections caused by multi-resistant *P. aeruginosa* because it has a broad spectrum antibacterial activity and due to their stability for most β-lactamases. Recently, show increased the rate of resistance for carbapenem drug activity, extraordinary resistance to *P. aeruginosa* was observed against imipenem (25%) and meropenem (21.2%). Previously, some research was reported, the carbapenem resistance 14.8% clinical isolates of *P. aeruginosa* from Najaf hospitals, 9.2% from Al-Nasseryia hospitals (18). The mechanisms of resistance to carbapenem expression of β-lactamases and efflux pumps, as well as alterations in PBP and porin loss, are all associated with carbapenem resistance in Gram-negative rods (19). Relatively, this study regarding the dissemination of carbapenem-hydrolyzing enzymes in Iraq, in the most detail by holding the respective *blue*-gene carried by 13 carbapenem-resistant *P. aeruginosa* clinical isolates and to learn their antibiotic resistance relationship. PCR analysis revealed that 9 (69.2%) isolates possessed at least one carbapenemase *bla*-gene (Table 3). MBLs (Class B) are the major mechanism of resistance to imipenem and meropenem, which include the IMP and VIM β-lactamase genes in addition to the AIM-1, NDM-1, GIM-1, SPM-1, and SIM-1 β-lactamases (20). The most common MBL identified in carbapenem-resistant *P. aeruginosa* in Iraq. So far, found 11.1% and 17.2% of carbapenems resistant *P. aeruginosa* isolates were positive for bla_{VIM} gene (17,18). Moreover, VIM-positive *P. aeruginosa* were found as reasons for many nosocomial infections in Saudi Arabia (21), the USA (22) Russia (23), and Turkey (24). In this regard the present study, 5 (38.5%) carbapenem resistant isolates were MBL

Table 1. Oligonucleotide primers used for detection of genes encoding carbapenemases in *P. aeruginosa*

Primer	Nucleotide Sequences (5'-3')	Gene detected	Amplicon Size(bp)	Reference
IMP	F: TTG ACA CTC CAT TTA CDG*	*bla*$_{IMP}$		(14)Dallenne *et al.*,
	R: GAT YGA GAA TTA AGC CAC YCT		139	2010
VIM	F: GAT GGT GTT TGG TCG CAT A	*bla*$_{VIM}$		(14)Dallenne *et al.*,
	R: CGA ATG CGC AGC ACC AG		390	2010
KPC	F:ATGTCACTGTATCGCCGTCT	*bla*$_{KPC}$		(15)Schechner *et*
	R:TTTTCAGAGCCTTACTGCCC		892	*al*., 2009
OXA-40	F: CACCTATGGTAATGCTCTTGC	*bla*$_{OXA-40}$		(16)Chelsie and
	R: GTGGAGTAACACCCATTCC		491	Nancy, 2013
OXA-50	F: AATCCGGCGCTCATCCATC	*bla*$_{OXA-50}$		(17)Girlich
	R: GGTCGGCGACTGAGGCGG		869	*et al*., 2004

Table 2. Summarizes the susceptibility testing results of the isolates *P. aeruginosa*

ANTIBIOTIC	NO. (%) OF ISOLATES (N=52)		
	Resistant	Intermediate	Sensitive
Ticarcillin	26 (50.0)	0 (0)	26 (50.0)
Pipracillin	21 (40.4)	0 (0)	31 (59.6)
Ticarcillin-clavulanic acid	23 (44.2)	0 (0)	29 (55.8)
Piperacillin-tazobactam	19 (36.5)	2 (3.8)	31 (59.7)
Ceftazidime	19 (36.6)	0 (0)	33 (63.4)
Cefepime	16 (30.8)	6 (11.5)	30 (57.7)
Imipenem	13 (25.0)	12 (23)	27 (52)
Meropenem	11 (21.2)	2 (3.8)	39 (75)
Amikacin	12 (23.0)	3 (5.8)	37 (71.2)
Gentamicin	23 (44.2)	3 (5.8)	26 (50)
Tobramycin	17 (32.7)	1 (1.9)	34 (65.4)
Ciprofloxacin	22 (42.3)	2(3.8)	28 (53.9)
Pefloxacin	23 (44.2)	3 (5.8)	26 (50)
colistin	2 (3.8)	0 (0)	50 (96.2)

Table 3. Distribution of class A, B, and D carbapenemase among carbapenem-resistant *P. aeruginosa* isolates (n=13)

Type of genes	No.(%) of positive isolates	Type of multi-antibiotics resistant
bla$_{OXA-50}$+ *bla*$_{VIM}$	4 (30.8)	XDR
bla$_{OXA-50}$	3 (23.0)	XDR
bla$_{KPC}$	1 (7.7)	XDR
bla$_{VIM}$	1 (7.7)	XDR
No amplification	4 (30.8)	(3) XDR
		) MDR

* XDR; Extensive drug resistance. MDR; Multi-drug resistance.

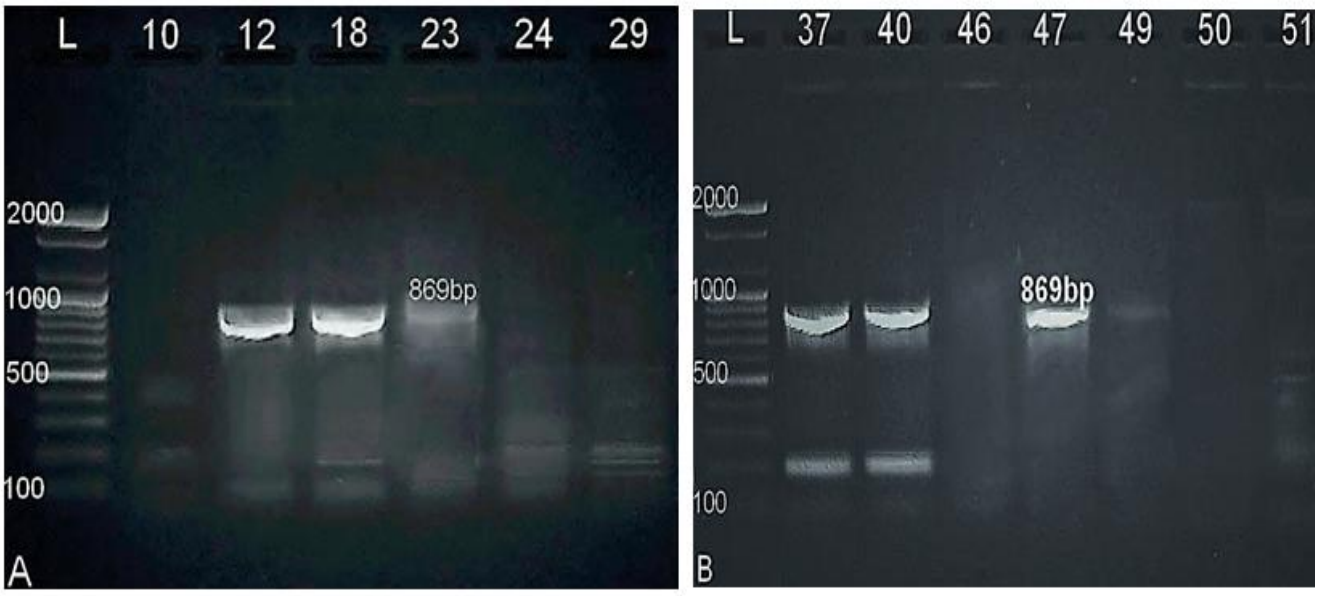

Figure (2 -A and B). Agarose gel electrophoresis for the amplification products of *P. aeruginosa* isolates that amplified using *bla*$_{OXA-50}$ gene primers with product 869bp. Lane (L), DNA molecular size marker (2000bp ladder), Lanes (12, 18, 23, 37, 40, 47 and 49) show positive results with *bla*$_{OXA50}$ gene

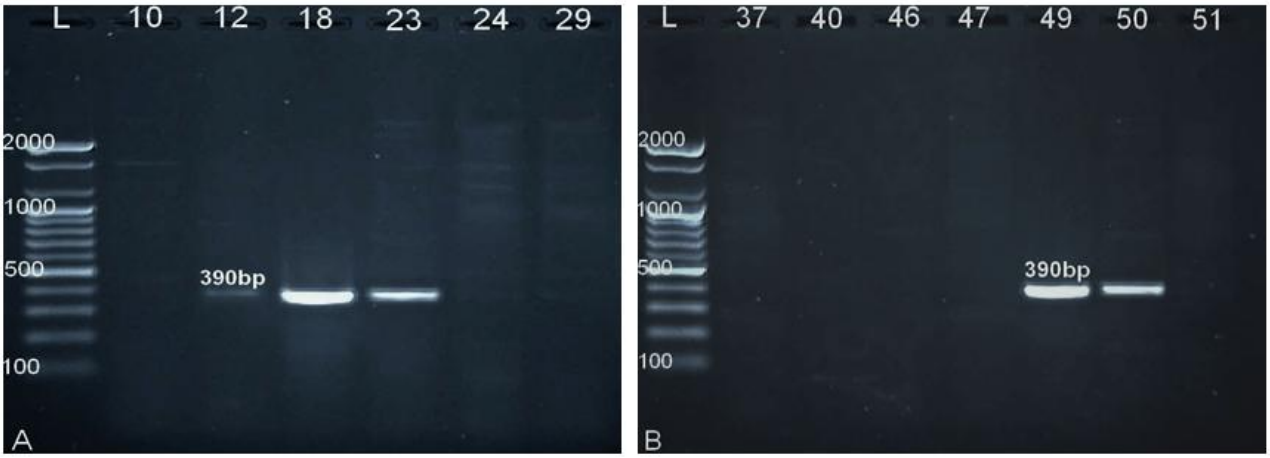

Figure 3. A and B. Agarose gel electrophoresis for the amplification products of *P. aeruginosa* isolates that amplified using bla_{VIM} gene primers with product 390bp. Lane (L), DNA molecular size marker (2000-bp ladder), Lanes (12, 18, 23, 49 and 50) show positive results with bla_{VIM} gene.

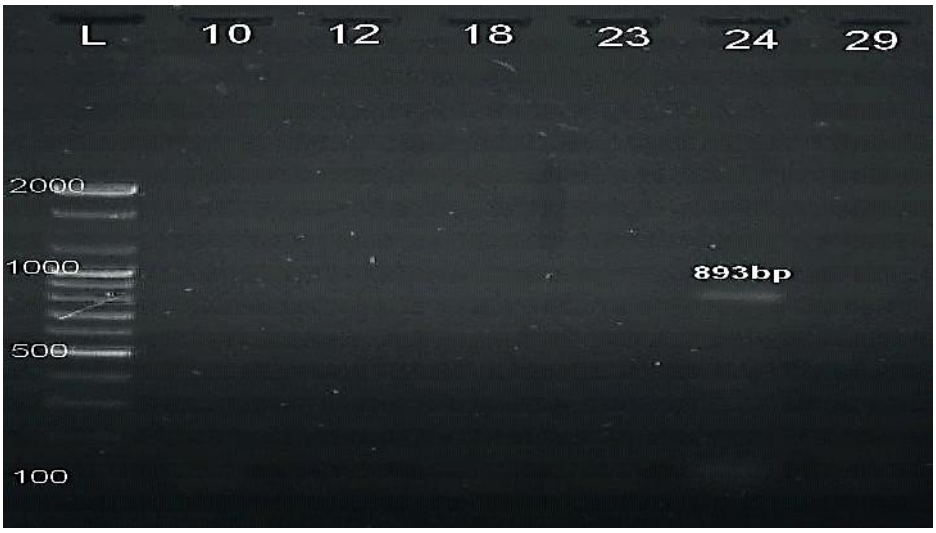

Figure 4. Agarose gel electrophoresis for the amplification products of *P. aeruginosa* isolates that amplified using: bla_{KPC} gene primers with product 893bp. Lane (L), DNA molecular size marker (2000-bp ladder), Lane (24) show positive results with bla_{KPC} gene

producers, showed high-level resistance to imipenem and meropenem and all carried the bla_{VIM} gene. The VIM was identified to be carried on gene cassettes. The cassettes are inserted into class 1 integron. Integron-located resistance genes offer them with an increased potential for distribution and expression (25). In Iraq and for the first time, our assay recorded one clinical isolate of *P. aeruginosa* that produced class A carbapenemase, KPC. Previously, The first characterized KPC-producing *P. aeruginosa* isolate was detected in Colombia and reported in 2007, and later in Puerto Rico, in the United States, Greece, Italy and in China (26).OXA-50 (Class D carbapenemase) commonly presents on the chromosomes and seem to be naturally encoded in strains of *P. aeruginosa* (6). The present result showed an unexpected proportion of *P. aeruginosa* producing OXA-50 in (53.8%) seven isolates of the 13 carbapenem resistant isolates were positive by PCR for the bla_{OXA-50}. Obviously, the OXA β-lactamase within carbapenemases have the capability to confer carbapenem resistance and may already the bacteria have an intrinsic resistance to numerous classes of antibiotic. This is particularly clear in the *Acinetobacter* spp. strains that has been recognized from soldiers and civilian populations returning from military duty in the Iraq (27), and recently recognized in two isolates from Najaf hospitals (4). However, co-production of OXA-50 and VIM carbapenemase was detected in 4 (30.8%) isolates. Therefore, indications unusefull for treatment of burn infections caused by these bacteria which because of a therapeutic uncontrolled in which all β-lactam drugs and lead to much more serious implications. In four carbapenem resistant isolates tested in this study, no carbapenemases were identified. The reduced susceptibility to carbapenems in these isolates was possible to be due to others reasons such as efflux pump, Porin loss, rise AmpC-production, or can be due to other types of carbapenemase are present and not tested in the current search. Most acquired MBL genes are carried on mobile gene cassettes inserted in integrons, related to mobile DNA elements (plasmids and transposons) like those for OXA β-lactamases, those integrons frequently harbored other gene

cassettes carrying resistance determinants for disinfectants, other antibiotic classes (28). That showed an overall cross-resistance to other antibiotics was common among carbapenem resistance *P. aeruginosa* , which ranged from 55.6% to 100% and all were susceptible to colistin. Furthermore, all these isolates were found to be XDR.

CONCLUSION

Our study has shown the extended spectra of resistance activity were found among nosocomial *P. aeruginosa* strains. Plasmid mediated VIM enzymes and chromosomally encoded ESBLs (OXA-50) have been discovered, these enzymes may represent potential threats patients with burn infections in Al-Najaf hospitals, since gene-capture units may mobilize them onto plasmids and may enhance their dissemination, to taking into account the threat of co-resistance, therefore clinical efforts for early recognition of acquired β-lactamase producing strains and rigorous infection control measures should be underscored.

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